Case Report Fatal vancomycin- and linezolid-resistant Enterococcus faecium sepsis in a child undergoing allogeneic haematopoietic stem cell transplantation for beta-thalassaemia major

Marco Fossati,1 Barbara Cappelli,1,2 Erika Biral,1,2 Robert Chiesa,1,2 Alessandra Biffi,1,2 Cristina Ossi,3 Matteo Moro,4 Daniela Maria Cirillo,5 Massimo Clementi,3,6 Clara Soliman,1 Fabio Ciceri,1 Maria Grazia Roncarolo,1,2,6 Luca Fumagalli7 and Sarah Marktel1,2

Correspondence Barbara Cappelli barbara.cappelli@hsr.it
1Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy
2San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy
3Microbiology Laboratory, San Raffaele Scientific Institute, Milan, Italy
4Chief Medical Office, San Raffaele Scientific Institute, Milan, Italy
5Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Milan, Italy
6Vita-Salute San Raffaele University Medical School, Milan, Italy
7Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

Received 29 December 2009
Accepted 16 March 2010

Introduction
Patients undergoing myeloablative haematopoietic stem cell transplantation (HSCT) are at increased risk of severe and life-threatening infections, in relation to severe neutropenia, disruption of mucosal surfaces and deep impairment of humoral and cellular immunity. In addition, primary or secondary graft failure can lead to prolonged pancytopenia. Despite the introduction of new antibiotic compounds, infections still represent one of the major causes of death in HSCT recipients. Enterococcus species, facultatively anaerobic, Gram-positive cocci, are commensals of the gastrointestinal and vaginal tracts, as well as of the oral cavity. Enterococcus species show a high level of intrinsic resistance to antibiotics, including cephalosporins, penicillinase-resistant penicillins, cotrimoxazole and clindamycin. Low-level intrinsic resistance to aminoglycosides occurs in most strains. The incidence of vancomycin-resistant Enterococcus (VRE) bloodstream infections related to the increasing use of antibiotics has increased worldwide (McDonald, 2006). In the last two decades, particularly virulent strains of VRE have emerged in nosocomial infections (Deshpande et al., 2007). Thus therapeutic options for serious VRE infections are limited (Cetinkaya et al., 2000). New antimicrobials active against VRE such as linezolid and quinupristin/dalfopristin have been developed, with response rates of approximately 70 % (Gonzales et al., 2001). Recently, tigecycline and daptomycin have been introduced in clinical practice, while other antimicrobials such as oritavancin are in the late stage of development (Jenkins, 2007). However, resistance has already been reported to some of these agents (Herrero et al., 2002). Linezolid-resistant VRE (LR-VRE) has been described sporadically in adult patients (Gonzales et al., 2001; Jenkins, 2007; Seedat et al., 2006; Cian et al., 2009). LR-VRE has not yet been reported in paediatric patients to our knowledge. We report the first case of a paediatric patient developing a fatal LR-VRE sepsis in spite of daptomycin treatment following HSCT for thalassaemia major.

Case report
An 11-year-old Syrian child with high risk/class III beta-thalassaemia major was referred to our Institution to receive a HSCT. She was severely iron overloaded as evidenced by hepatomegaly, liver fibrosis, high levels of...
serum ferritin and liver iron concentration. She received a first HSCT from her human leukocyte antigen (HLA)-identical brother in November 2007 but unfortunately experienced primary graft failure with persistent pancytopenia in spite of reinfusion of autologous marrow as a rescue. To treat the irreversible pancytopenia (absolute neutrophil count 1000 mm$^{-3}$; platelets 5000 mm$^{-3}$), the patient was offered a second HSCT. The conditioning regimen consisted of strong immunosuppression and limited myeloablation to reduce regimen-related toxicity: anti-thymoglobulin (8 mg kg$^{-1}$), fludarabine (150 mg m$^{-2}$), thiotepa (10 mg kg$^{-1}$), rituximab (375 mg m$^{-2}$) and cyclophosphamide (160 mg kg$^{-1}$). Graft-versus-host disease prophylaxis consisted of short methotrexate, cyclosporin A and methylprednisolone. Anti-infective prophylaxis consisted of ciprofloxacin, nebulized pentamidine, acyclovir and liposomal amphotericin B 1 mg kg$^{-1}$ per day. On admission, microbiological surveillance consisting of pharyngeal, nasal and anal swabs was negative. The patient was hosted in a single room with a private toilet. The standard approach to the patient consisted of hand washing before and after access to the room and during patient care as recommended by the Centers for Disease Control and World Health Organization (CDC, 2002; WHO, 2009), in addition to face masks and single-use gowns as per hospital infection control policy. All doctors, nurses and visitors were trained by the incharge nurse and given a ward information booklet before admission to the Transplantation Unit as recommended by the hospital infection control policy. A central venous catheter was placed in the right femoral vein due to refractory thrombocytopenia contraindicating more invasive approaches. In December 2008, the patient received an unmanipulated HLA-identical HSCT from her brother (second HLA identical HSCT, 10 $\times$ 10$^6$ CD34$^+$ kg$^{-1}$). Unfortunately, the bone marrow aspiration performed on day 21 showed 0% donor chimerism suggesting complete transplant rejection. In consideration of the poor prognosis as rescue therapy, the patient underwent an infusion of haploidentical non-manipulated peripheral blood stem cells from her father (first haplo HSCT, 12 $\times$ 10$^6$ CD34$^+$ kg$^{-1}$). Unfortunately, we did not observe any haematopoietic recovery, and the bone marrow aspiration, performed 18 days later, showed 0% donor chimerism. Cryopreserved stem cells previously collected from the father (second haplo HSCT, 6.8 $\times$ 10$^6$ CD34$^+$ kg$^{-1}$) were therefore given as a last effort to rescue the patient from irreversible aplasia. She never recovered haemopoiesis.

From the infectious point of view, on day 13 after the second HSCT, the patient developed high fever and a high C-reactive protein level (168 mg l$^{-1}$). She was started on ceftazidime, amikacin and vancomycin and the femoral central venous catheter was removed. Streptococcus mitis
(Fig. 1) was isolated from blood culture. The infection resolved 4 days later. A peripherally inserted central catheter was placed in the left radial vein. On day 34 from the second HSCT, a positive Aspergillus antigen of 0.68 (galactomannan test, normal value <0.5) was detected, rising to 3.7 on day 41 when i.v. voriconazole 15 mg kg\(^{-1}\) was introduced. The infection spread to the respiratory tract and central nervous sytem as suggested by a chest CT and a brain MRI, therefore caspofungin (50 mg m\(^{-2}\)) was added. The patient remained aplastic and on day 50 developed fever associated with clinical signs of sepsis and increased C-reactive protein. The patient did not develop diarrhoea or vomiting. She was at that time on voriconazole, caspofungin, levofloxacin, linezolid and meropenem, which was empirically changed to piperacillin/tazobactam. Blood cultures as well as pharyngeal and anal swabs tested positive for *Enterococcus faecium*. A transthoracic echocardiogram was negative for endocarditis and a chest CT was unchanged. The antibiotic sensitivity test showed a strain resistant to ampicillin/sulbactam (MIC \(\geq 32 \mu g \text{ ml}^{-1}\)), ciprofloxacin (MIC \(\geq 8\)), clindamycin (MIC \(\geq 8\)), erythromycin (MIC \(\geq 8\)), imipenem (MIC \(\geq 16\)), teicoplanin (MIC \(\geq 96\)), cotrimoxazole (MIC \(\geq 320\)), vancomycin (MIC \(\geq 256\)), levofloxacin (MIC \(\geq 8\)), quinupristin/dalfopristin (MIC=4) and linezolid (MIC \(\geq 8\)), sensitive to tetracycline (MIC=0.25), daptomycin (MIC=1.5) and tigecycline (MIC<0.12), and intermediate to chloramphenicol. She was started on oral doxycycline 2.5 mg kg\(^{-1}\) on day 52, but due to difficulties in administering the oral formulation and unavailability of the i.v. formulation in Italy, this was discontinued 2 days later and i.v. daptomycin 4 mg kg\(^{-1}\) was introduced. Unfortunately, severe pancytopenia persisted in the child and she died 63 days post second HSCT. *E. faecium* was isolated from blood cultures until exitus. After the first LR-VRE isolation, the standard approach to the patient was integrated with specific contact precautions consisting of single-use gowns, cap, gloves and overshoes before entering the room as per local infection control policy. Epidemiological investigation with environmental sampling and active screening (rectal and pharyngeal swabs) of the five patients in the Transplantation Unit was carried out. No growth was observed in the environment, but one patient was colonized by *E. faecium* with the same antibiotic resistance pattern. This patient was not previously treated with linezolid. The molecular typing of the two isolates performed by PFGE (Fig. 2) and by VanA PCR (Fig. 3) showed an identical pattern. Specific contact precautions described above were also applied for this patient until colonization clearance. Following isolation of the multiresistant bacterium, rectal and pharyngeal swabs were collected weekly for 2 months and antimicrobial prophylaxis with ceftriaxone was discontinued to avoid further resistances. Probiotics were started to re-establish the normal intestinal flora. The patient had a good graft function, remained asymptomatic, no specific antimicrobial therapy was started and after 2 months he achieved spontaneous colonization clearance. A retrospective analysis of the PFGE patterns of clinically significant VRE strains isolated from inpatients showed that strains with an indistinguishable PFGE pattern have been occasionally isolated in the Transplantation Unit since April 2008 but not in other hospital wards.

**Discussion**

Patients undergoing HSCT experience a variable period of severe aplasia before engraftment and are particularly exposed to severe invasive infections. The improvement of

![Fig. 2. PFGE patterns of the two LR-VRE isolates following *SmaI* restriction and separation with a CHEF-DRIII apparatus (Bio-Rad) (Miranda et al., 1991). PFGE was performed in 0.5× TBE buffer at 14 °C. The pulsed-field parameters were 20 h at 6 V cm\(^{-1}\) (200 V), with switch times ramped from 5 to 35 s. Cluster analysis was performed with the software InfoQuest FP (optimization 0.5, tolerance 0.5). *Staphylococcus aureus* NCTC 8325 was used as molecular mass marker. VRE 1-09 represents the index case; VRE 2-09 represents the colonized patient.](http://jmm.sgmjournals.org/)

![Fig. 3. Confirmation of the VanA phenotype by PCR using the primers and protocol of Dutka-Malen et al. (1995). Lanes: 1, VRE 1; 2, VRE 2; 3, molecular mass marker.](http://jmm.sgmjournals.org/)
supportive care in transplantation units has led to a better prevention and treatment of infections. However, multiple factors have contributed to the selection of Gram-positive micro-organisms as the dominant pathogens, such as the use of antibacterial prophylaxis with quinolone derivates, more aggressive cytoreductive therapies promoting intestinal translocation, and the employment of intravascular devices. Gram-positive pathogens accounted for 65% of bacteriae- nias in a survey of 24,000 cases in 49 US hospitals (Wisplinghoff et al., 2004). Few cases of LR-VRE treated with tigecycline and daptomycin have been described in adults, suggesting the rapid emergence of these organisms (Gonzales et al., 2001; Seedat et al., 2006; Bonora et al., 2006). Enterococcus species are also responsible for considerable morbidity and mortality in paediatric patients (Han et al., 2009), but cases of LR-VRE have not been described yet to our knowledge. The patient in this report was at particular high risk because of advanced thalassaeemia major with organ dysfunction, severe iron overload and long-standing neutropenia following graft failure of the first HSCT. During the second HSCT, in spite of aggressive immunosuppression, she experienced primary graft failure with irreversible pancyto- penia requiring prolonged and numerous courses of wide-spectrum anti-infective treatment. Eventually, a LR-VRE isolate grew following this selective pressure. As suggested by recent reports and according to the sensitivity spectrum, daptomycin was preferred to tigecycline, for which few reported data are available (Jenkins, 2007; Pankey et al., 2005; Ardura et al., 2007). The choice was also supported by the probable fungal aetiology of the concomitant pneumoniae as suggested by a galactomannan test and imaging studies. Daptomycin was continued at a therapeutic dosage until exitus, 9 days later. This antibiotic therapy failed to sterilize blood cultures probably due to the short duration of treatment, irreversible graft failure and overwhelming multi-organ failure. Moreover, in spite of all the appropriate isolation measures routinely in place in the ward, we documented one case of transmission of the LR-VRE. The transmission was most likely healthcare-worker-related as several environmental swabs in the patient’s room were negative. Fortunately this did not result in morbidity for the colonized patient because of good haemopoietic recovery at the time of infection.

In conclusion, paediatric patients can also develop VRE resistant to new antibiotics under selective pressure. In severely compromised patients, the optimal treatment has yet to be determined. In this case, daptomycin was chosen in consideration of the prominent bloodstream infection, in spite of limited paediatric experience. Furthermore, the importance of isolating patients with multidrug-resistant organisms should be underlined, in order to minimize the spread of these life-threatening infections.

References


